PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 11 May 2000 (11.05.00)	
International application No. PCT/US99/20942	**Applicant's or agent's file reference** UMDNJ-31060
International filing date (day/month/year) 13 September 1999 (13.09.99)	**Priority date** (day/month/year) 14 September 1998 (14.09.98)
Applicant DINMAN, Jonathan, D. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

24 February 2000 (24.02.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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15

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference UMDNJ-31060	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/20942	International filing date *(day/month/year)* 13 September 1999 (13.09.1999)	Priority date *(day/month/year)* 14 September 1998 (14.09.1998)
International Patent Classification (IPC) or national classification and IPC IPC(7): C12N 15/11, 15/12 and US Cl.: 435/6, 440; 536/23.1, 23.5		
Applicant UNIVERSITY OF MEDICINE AND DENISTRY OF NEW JERSEY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand 24 February 2000 (24.02.2000)	Date of completion of this report 03 January 2001 (03.01.2001)
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Dorothea Laurence for John S. Brusca, Ph.D. Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/20942

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed.

☒ the description:
pages 1-22 as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the claims:
pages 23-25, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the drawings:
pages 1, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/20942

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☒ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention is accordance with Rules 13.1, 13.2 and 13.3 is

- ☒ complied with.
- ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claim(s) 1-6, drawn to a method of identification of a ribosomal frameshift sequence.

Group 2, claim(s) 7-18, drawn to a method of modulating gene expression.

The inventions listed as Groups 1 and 2 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature of Group 1 is a method of identifying a nucleic acid sequence involved in ribosomal frameshifting. The special technical feature of Group 2 is a method of regulating expression of a gene by modulating the frequency of ribosomal frameshifting. Therefore the two groups have different special technical features.

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☒ all parts.
- ☐ the parts relating to claims Nos. ____

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/20942

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-6, 10-18	YES
	Claims 7-9	NO
Inventive Step (IS)	Claims 10-18	YES
	Claims 1-9	NO
Industrial Applicability (IA)	Claims 1-18	YES
	Claims NONE	NO

2. CITATIONS AND EXPLANATIONS (Rule 70.7)

Claims 1-6 lack an inventive step under PCT Article 33(3) as being obvious over Honda et al. in view of Lee et al. in view of Scheffter et al.

The claims are drawn to methods for identifying ribosomal frameshift sequences in nucleic acid sequences, and systems for performing the method.

Honda et al. shows ribosomal frameshift sites in the introduction that meet the limitations of the claimed invention. Honda et al. shows ribosomal frameshift sites in HIV in figure 1, and variants therof in figure 2 that reduce the frameshift efficiency. Honda et al. does not show a method for identifying frameshift sequences.

Lee et al. shows a method and computer system that detects ribosomal frameshift sites on page 22 and figure 1B.

Scheffter et al. shows a method and computer system on page 483 and figure 3 that detects ribosomal frameshift sites.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to detect the ribosomal frameshift motifs taught by Honda et al. by use of the methods of Lee et al. and Scheffter et al. because Honda et al., Lee et al., and Scheffter et al. each show that ribosomal frameshift sequences are important in a number of different viral life cycles.

Claims 7-9 lack novelty under PCT Article 33(2) as being anticipated by Matsufuji et al.

Matsufuji et al. shows in Figure 2 and throughout stimulation of expression of a rat antizyme gene by induction of ribosomal frameshifting upon addition of a polyamine.

Claims 10-18 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest modulation of expression of the recited genes by ribosomal frameshifting, or methods of treating disease by modulation of ribosomal frameshifting.

---------- NEW CITATIONS ----------

DINMAN et al. Peptidyl-transferase inhibitors have antiviral properties by altering programmed -1 ribosomal frameshifting efficiencies: Development of model systems. Proc. Natl. Acad. Sci. USA. June 1997, pages 6606-6611, especially page 6609.

DINMAN et al. Translating old drugs into new treatments: ribosomal frameshifting as a target for antiviral agents. Trends in Biotechnology. April 1998, pages 190-196, especially pages 195-196.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

The description is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 5 because it fails to adequately enable practice of the claimed invention because: .

Eight factors to be considered in a determination of undue experimentation include: (a) the quantity of experimentation necessary; (b) the amount of direction or guidance presented; (c) the presence or absence of working examples; (d) the nature of the invention; (e) the state of the prior art; (f) the relative skill of those in the art; (g) the predictability of the art; and (h) the breadth of the claims.

In considering the factors for the instant claims:

a) In order to practice the claimed invention one of skill in the art must treat a disease by increasing or decreasing ribosomal frameshifting. For the reasons discussed below, there would be an unpredictable amount of experimentation required to practice the claimed invention.

b) The description discusses the ability of anisomycic and sparsomycin to regulate ribosomal frameshifting. The description does not present specific guidance to treat diseases by modulation of ribosomal frameshifting.

c) The description does not present working examples of treatment of disease by modulation of ribosomal frameshifting.

d) The invention is drawn to treatment of disease by modulation of ribosomal frameshifting.

e) Dinman ('97) shows in figure 2 and througout that anisomycin decreases ribosomal frameshifting and that sparsomycin increases ribosomal frameshifting. Dinman ('97) shows in figure 3 that anisomycin and sparsomycin treatment result in the loss of a non-lethal virus from yeast cells. Dinman ('98) reviews ribosomal frameshifting and shows in Table 1 that many pathogenic viruses require ribosomal frameshifting in their life cycle. Dinman ('98) speculates on pages 195-196 that agents that modulate ribosomal frameshifting may be useful as therapeutic agents to treat viral diseases. The prior art does not show treatment of diseases caused by viruses or other pathogenic agents with agents that produce a therapeutic effect by modulation of ribosomal frameshifting.

f) The skill of those in the art of molecular biology is high.

g) The prior art does not show or predict the possibility of using agents that modulate ribosomal frameshifting to treat disease.

h) The claims are broad in that they require agents that modulate ribosomal frameshifting to be used to treat disease.

The skilled practitioner would first turn to the instant description for guidance in practicing the claimed therapeutic method, however no such guidance is present in the instant description. As such, the skilled practitioner would turn to the prior art for such guidance, however Dinman ('97) and Dinman ('98) also fail to provide such guidance. Finally said practitioner would turn to trial and error experimentation without guidance from the instant discription or the prior art. Such represents undue experimentation.

Claims 17 and 18 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because practice of the claimed invention is not enabled as required under PCT Rule 5.1(a) for the reasons set forth in the immediately preceding paragraph.

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Published
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Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: RIBOSOMAL FRAMESHIFT TARGETS

(57) Abstract

Sequences involved in ribosomal frameshifting have been discovered in mammalian genes. Methods of identifying ribosomal frameshift sequences in mammalian genes are disclosed. Methods of regulating gene expression by modulating ribosomal frameshifting are disclosed.

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RIBOSOMAL FRAMESHIFT TARGETS

Background of the Invention

Maintenance of correct reading frame during translation of mRNA is fundamental to the integrity of the translation process and, ultimately, to cell growth and viability. However, a number of cases have been identified in which translating ribosomes are directed to shift reading frames, a phenomenon referred to as "programmed ribosomal frameshifting". Most of these ribosomal frameshift events have been observed in RNA viruses. Families of mammalian viruses in which ribosomal frameshifting has been observed include retroviruses, coronaviruses, toroviruses, arteriviruses, astroviruses, and paramyxovirus. Plant viruses in which frameshifting has been observed include tetraviruses, and tombusviruses. In fungi, ribosomal frameshifting has been observed in the totiviruses and many retrotransposable elements. Among bacteriophages, ribosomal frameshifting has been documented in T7 and λ. Viral frameshifting events typically produce fusion proteins in which the N- and C-terminal domains are encoded by two distinct, overlapping open reading frames. Ribosomal frameshifting in viruses determines the stoichiometric ratio of structural (Gag) to enzymatic (Gag-pol) proteins, and plays a critical role in viral particle assembly. The study of these ribosomal frameshifts has been important both because of their critical role in viral morphogenesis, and because of the information they provide about the mechanisms by which reading frame is normally maintained.

The cis-acting sequences that promote efficient ribosomal frameshifting in the -1 (5') direction have been well characterized in several viral systems and it has been convincingly demonstrated that the basic molecular mechanisms governing programmed -1 ribosomal frameshifting are almost identical from yeast to humans. Two basic sequence elements are required to promote efficient levels of programmed -1 ribosomal frameshifting. The first sequence element is heptamer sequence, X XXY YYZ (wherein the 0-frame is indicated by spaces) called the "slippery site". The simultaneous slippage of ribosome-bound A- and P-site tRNAs by one base in the 5' direction still leaves their non-wobble bases correctly paired with the mRNA in the new reading frame. The second promoting element is usually a sequence that forms a defined RNA secondary structure, such as an RNA pseudoknot, located within 8 nucleotides 3' of the slippery site, and is thought to increase the probability that the ribosome will shift reading frame in the -1 direction. The number of ribosomes that shift frame is affected by a number of parameters, including the ability of the ribosome bound tRNAs to unpair from the 0-frame, the ability of these tRNAs to rebind to the -1 frame, the relative position of the RNA pseudoknot from the slippery site and the thermodynamic stability of the pseudoknot.

There are a few documented examples in which programmed ribosomal frameshifting is utilized by mRNAs of cellular origin. In *E. coli*, autoregulation of a programmed +1 ribosomal frameshift in the

prfB gene is required for the synthesis of Release Factor 2 (RF2) (Craigen and Caskey, 1986; Craigen et al., 1985; Donly et al., 1990a; Donly et al., 1990b), and a -1 ribosomal frameshift in the *dnaX* gene generates the DNA polymerase gamma subunit (Flower and McHenry, 1991; Blinkowa and Walker, 1990; Tsuchihashi and Kornberg, 1990). In eukaryotic mRNAs, programmed +1 ribosomal frameshifting has been demonstrated in genes encoding ornithine decarboxylase (ODC) Antizyme isolated from rat, mouse, *xenopus*, *drosophila* (Hayashi and Murakami, 1995; Ivanov et al., 1998; Kankare et al., 1997; Ichiba et al., 1995; Matsufuji et al., 1995; Rom and Kahana, 1994), and in the *EST3* gene of *S. cerevisiae* (Lundblad and Morris, 1997). In mammalian cells, the control of ribosomal frameshifting efficiency is autoregulated by ODC Antizyme protein levels (Craigen and Caskey, 1986; Craigen et al., 1985; Donly et al., 1990a; Hayashi and Murakami, 1995; Matsufuji et al., 1995). In yeast cells which lack ODC Antizyme, high concentrations of putrecine and consequently low concentrations of spermidine promote increased efficiencies of frameshifting in the +1 direction (Balasundaram et al., 1994b; Balasundaram et al., 1994a). Thus, the regulation of polyamine biosynthesis demonstrates how programmed ribosomal frameshifting may be used by eukaryotic cellular genes as a post-transcriptional regulatory mechanism. Although there are no known examples of eukaryotic cellular mRNAs which utilize programmed -1 ribosomal frameshifting, certain observations suggest that this mechanism may also be biologically relevant for these cells as well. Certain yeast strains harboring chromosomal mutations which increase the efficiency of -1 ribosomal frameshifting (*mof* = *m*aintenance *of* *f*rame) show cellular defects as well, *e.g.* temperature sensitive cell cycle growth arrest, temperature-sensitive mating defects, mitochondrial defects, sensitivity to translational inhibitors, inability to degrade nonsense mRNAs, and slow growth phenotypes (Cui et al., 1996; Dinman and Wickner, 1992; Dinman and Wickner, 1994). These observations suggest that -1 ribosomal frameshifting may play a role in the regulation of cellular gene expression, and that changes in the efficiency of -1 ribosomal frameshifting may affect cell growth and replication.

Based on the hypothesis that biological systems tend to conserve and use functional molecular regulatory mechanisms, a computer search program was designed to identify consensus -1 ribosomal frameshift signals in large DNA databases. It was found that consensus -1 ribosomal frameshift signals occur with frequencies significantly greater than random in these databases. It was also demonstrated that one of the predicted -1 ribosomal frameshift signals, occurring at the 5' end of the yeast Rasl mRNA, promotes efficient levels of -1 ribosomal frameshifting in the yeast *S. cerevisiae*.

Summary of the Invention

In accordance with the present invention, it has been discovered that gene sequences which have the frameshifting sequences exist in many organisms other than viruses. Frameshifting sequences have been newly identified in numerous yeast, avian, and mammalian sequences.

A computer search was designed to search for consensus -1 ribosomal frameshift signals (motif hits) present in the EMBL virus, *Saccharomyces cerevisiae*, human mRNA, cDNA and Expressed Sequence Tag (EST) databases. These searches found that potential -1 ribosomal frameshifting signals occur at frequencies greater than one order of magnitude above random chance. This result provides strong theoretical evidence for the existence of a subset of cellular genes which are regulated at the translational level by -1 ribosomal frameshifting in eukaryotes, and that this post transcriptional regulatory mechanism is widely used by many different families of viruses as well.

The present invention provides a method of identifying a nucleic acid sequence involved in ribosomal frameshifting. The method comprises 1) searching a database of gene sequences to identify sequences which contain the sequence XXX YYY Z , wherein XXX represents GGG, AAA, TTT or CCC, YYY represents AAA or TTT, Z represents A, T, or C and wherein XXXYYYZ is not AAAAAAA or TTTTTTT; and 2) further searching among those sequences identified in step 1 for a sequence encoding a pseudoknot structure which is within eight nucleotides of the sequence identified in step 1.

The present invention also provides a method of identifying a nucleic acid sequence involved in ribosomal frameshifting, comprising the steps of selecting a gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC; selecting said gene sequence having an adjacent sequence of nucleotides from the group of AAA and TTT; selecting said gene sequence having a nucleotide from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides; excluding said gene sequence wherein said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A; excluding said gene sequence wherein said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T; searching for an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

The present invention further provides a system for identifying a nucleic acid sequence involved in ribosomal frameshifting, the system comprising access means for accessing a database of gene sequences; selection means for selecting a particular gene sequence from said database of gene sequences, said particular gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC, an adjacent sequence of nucleotides from the group of AAA and TTT, a nucleotide from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides, wherein said particular gene sequence is excluded from selection when said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A and said particular gene sequence is excluded from selection when said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T; pseudoknot search means for locating an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

The present invention also provides a method of regulating expression of a mammalian gene comprising modulating the frequency of ribosomal frameshifting during translation of messenger RNA.

Brief Description of the Drawings

Figure 1: Consensus programmed -1 ribosomal frameshift signal.

Figure 2: Conservation of two frameshift signals in homologous genes from different organisms.

Detailed Description of the Invention

The present invention provides a method of identifying a nucleic acid sequence involved in ribosomal frameshifting. The method comprises searching a database of gene sequences to identify nucleic acid sequences which contain a slippery site and a pseudoknot structure associated with frameshifting. The method comprises first searching for a slippery site, which is identified by the sequence XXX YYY Z, wherein XXX represents GGG, AAA, TTT or CCC; YYY represents AAA or TTT; Z represents A, T, or C; and wherein XXXYYYZ is not AAAAAAA or TTTTTTT. Further searching is conducted among those sequences containing a slippery site for a sequence encoding a pseudoknot structure which is within eight nucleotides of the slippery site sequence.

The slippery site may have any of the following nucleic acid sequences: GGG AAA A, GGG AAA T, GGG AAA C, AAA AAA T, AAA AAA C, TTT AAA A, TTT AAA T, TTT AAA C, CCC AAA A, CCC AAA T, CCC AAA C, GGG TTT A, GGG TTT T, GGG TTT C, AAA TTT A, AAA TTT T, AAA TTT C, TTT TTT A, TTT TTT C, CCC TTT A , CCC TTT T, and CCC TTT C.

The present invention also provides a method of identifying a nucleic acid sequence involved in ribosomal frameshifting, comprising the steps of selecting a gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC; selecting said gene sequence having an adjacent sequence of nucleotides from the group of AAA and TTT; selecting said gene sequence having a nucleotide from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides; excluding said gene sequence wherein said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A; excluding said gene sequence wherein said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T; searching for an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

The present invention further provides a system for identifying a nucleic acid sequence involved in ribosomal frameshifting, the system comprising access means for accessing a database of gene sequences; selection means for selecting a particular gene sequence from said database of gene sequences, said particular gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC, an adjacent sequence of nucleotides from the group of AAA and TTT, a nucleotide

from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides, wherein said particular gene sequence is excluded from selection when said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A and said particular gene sequence is excluded from selection when said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T; pseudoknot search means for locating an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

Translation of any gene containing frameshift sequences, namely the slippery site and psuedoknot sequences, is potentially regulated by the ribosomal frameshifting mechanism. Consequently, translation of such a gene may be regulated by known methods of altering the frequency of frameshifting, for example, by use of drugs which affect the peptidyl transferase activity. Accordingly, the invention provides a method of regulating expression of a mammalian gene comprising modulating the frequency of ribosomal frameshifting during translation of messenger RNA. In accordance with the method, the frequency of frameshifting may be increased or decreased.

Computer search protocols.

The GenBank *Saccharomyces cerevisiae*, *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Gallus gallus*, *Sus scrofa*, *Drosophila melanogaster*, and Virus divisions, and 2 x 10^4 random sequences of 10^3 bases (G-C content = 50%) were searched using the following algorithmic structure:

Step 1: Search for XXXYYYZ (slippery site) where:

XXX = GGG, AAA, TTT or CCC

YYY = AAA or TTT

Z = A, T, or C

AND XXXYYYZ ≠ AAAAAAA or TTTTTTT.

Step 1 can be implemented by selecting a gene sequence having a sequence of nuceotides from the group of GGG, AAA, TTT and CCC; selecting the gene sequence having an adjacent sequence of nucleotides from the group of AAA and TTT; selecting the gene sequence having a nucleotide from the group of A, T and C, the nnucleotide adjacent to the adjacent of nucleotides; excluding the gene sequence wherein the sequence of nucleotides is AAA, the adjacent sequence of nucleotides is AAA and the nucleotide is A; and excluding the gene sequence wherein the sequence of nucletides is TTT, the adjacent sequence of nucleotides is TTT and the nucleotide is T.

Step 2: Search for a pseudoknot 3' of the XXXYYYZ slippery site motif using the GenoBase program. Further constraints placed on the pseudoknot were:

a. The pseudoknot must begin within 8 nucleotides (NT) of base Z;

b. Stem 1 must have a minimum length of 6 base pairs, containing no more than 1 mismatch, 1 insertion or 1 deletion;

c. Gap 1 (the gap between stem 1 and stem 2) can be no greater than 3 NT in length;

d. Stem 2 must have a minimum of 5 base pairs with only 1 insertion, deletion or mismatch allowed;

e. Gap 2 can be no greater than 3 NT in length;

f. Gap 3 is limited to 100 NT in length.

Step 3: Align motifs found in steps 1 and 2 with an open reading frame (ORF) of at least 50 codons, such that the first base in the slippery site (the first X) is in the third base of a codon. Further, searching in the 5' direction of the motif there must be an in-frame ATG codon before a translational termination signal (TAA, TAG, or TGA). Sequences that satisfied all of these criteria were defined as "motif hits".

Strains, media , genetic methods, and plasmid construction.

E. coli strain DH5 was used for plasmid preparations, and transformations of *E. Coli* and *S. Cerevisiae* were performed (Dinman and Wickner, 1992). YPAD and synthetic complete medium were prepared (Dinman and Wickner, 1994). The *S. cerevisiae* strain JD88 (*MATa ura3-52 lys2-801 ade2-10 trp1)* [L-AHNB] [M_1]) was used for *in vivo* measurements of -1 ribosomal frameshifting efficiencies as described in (Dinman and Wickner, 1992).

pJD160.0 is based on p314-JD86-ter (Cui et al., 1996), with the modification that it contains unique *Bam* HI, *Sma* I and *Kpn* I restriction endonuclease recognition sites 3' of the AUG start codon, and 5' of the *lacZ* gene. This is the 0-frame control plasmid. pJD160.-1 is identical to pJD160.0 except that lacZ is in the -1 frame with respect to the translational start site without any intervening frameshift signal. This is used to measure unprogrammed -1 ribosomal frameshifting. The frameshift signals from the yeast *RAS1* gene was amplified from genomic DNA by polymerase chain reaction (PCR) as described (Costa and Weiner, 1995) using the synthetic oligonucleotide primers shown in Table 1.

Table 1. Oligonucleotid Primers used in this study

Oligonucleotide Primer	Description
5' AAAG*AATTCC*GA**CATGCAGGGAAATCCAAATCAAC** 3' (SEQ ID NO:1)	RAS1 5' Eco RI.
5' CCCC*GGTACC***GTCATCGATGACAACTT** 3' (SEQ ID NO:2)	RAS1 3' Kpn I.

Italicized bases denote added restriction endonuclease recognition sites. Bold bases indicate gene sequence. Underlined bases were added 3' of the slippery site and 5' of the predicted mRNA pseudoknot forming region so that a -1 ribosomal frameshift will direct elongating ribosomes into the original reading frame.

Since the RAS1 frameshift signal is predicted to direct ribosomes into premature termination signals, two additional nucleotides were added in the spacer regions between the slippery sites and pseudoknots of these PCR products such that a -1 frameshift would re-direct ribosomes into the original reading frame. The PCR products were cloned into pJD160.0 to produce pJD160.RAS1. In this construct, a programmed -1 frameshift is required for in order for the *lacZ* gene to be translated.

RESULTS

The program is capable of finding known viral programmed -1 ribosomal frameshift signals.

As a positive control, the program was used to search all 36,556 loci of the GenBank virus division, revealing 1077 motif hits. The program identified almost all of the known viral -1 ribosomal frameshift signals including those that have been classically used to study programmed -1 ribosomal frameshifting. These include Mouse Mammary Tumor Virus, Feline Leukemia Virus, and Infectious Bronchitis Virus. As expected, the program was not able to identify the motif hit in Rous Sarcoma Virus because the Gaps 1 and 2 represented in Figure 1 are larger than allowed by the program. In addition, many motif hits were identified in families of viruses where -1 ribosomal frameshifting has not been described. For example, a frameshift motif appears to be well conserved in the E1B protein large T-antigen mRNA among the adenoviruses, and in the VP16 family of proteins in many of the herpesviruses.

Consensus motif hits occur at frequencies significantly greater than random in the genome databases.

If a subset of cellular genes utilize programmed -1 ribosomal frameshifting, then it may be assumed that the consensus frameshift motifs should be present in the genomes of many different species at frequencies significantly greater than random. To test this, the probability of the random occurrence of a motif hit was determined. The program was run twice against 10^4 randomly generated sequences of 10^3 bases. For technical reasons, the G:C content was set to 50%. This negative control found 41 motif hits in the first run and 42 in the second. Thus, the random frequency of motif hits is 83 per 2×10^7 bases. Searches of the large DNA databases revealed that motif hits occur with frequencies significantly greater than random (Table 2).

Table 2. Summary of search results.

Organism	# Bases Searched	# Motif Hits	Fold > Random
Random sequence	2.0×10^7	83	-
Saccharomyces cerevisiae (yeast)	1.2×10^7	260	5.22
Homo sapiens (human)	9.52×10^7	1055	2.67
Mus musculus (mouse)	2.13×10^7	320	3.62

Rattus norvegicus (rat)	1.14×10^7	103	2.18
Gallus gallus (chicken)	2.37×10^6	57	5.8
Sus scrofa (pig)	1.5×10^6	25	4.02
Drosophila melanogaster (fruitfly)	1.16×10^7	167	3.47
Viruses	3.7×10^7	1077	7.0

The results from the *S. cerevisiae* genome should provide the best estimate of the frequency of motif hits, because 1) it is complete, 2) it is on the same order of magnitude as the random control, 3) it contains the least amount of duplications, and 4) it was sequenced without reading-frame bias. Analysis of this dataset revealed 260 motif hits, approximately 5.2-fold more frequent than random. BLAST analysis revealed that 153 different recognized genes or CDS were represented. Since the yeast genome is estimated to contain approximately 5900 genes, these data suggest that at least 2.55% of the genes in the yeast genome contain at least one consensus programmed -1 ribosomal frameshift signal. Further, since the algorithm limited the size of gap1 and gap2 and disallowed slippery sites of TTTTTTT and AAAAAAA , the data probably represent an underestimate of the fraction of motif hits containing yeast genes.

Frameshift signals appear to be evolutionarily conserved between homologous genes in different species.

If a subset of cellular genes utilize programmed -1 ribosomal frameshifting, then specific frameshift signals would be evolutionarily conserved in homologous genes from different organisms. A preliminary comparison of the locations and structures of motif hits in homologous genes in the different databases reveals cases where nearly identical motif hits appear to be conserved. Two such examples, a comparison of Fibrillin 2 in human and mouse, and of the Sulfonurea Receptor in humans and rat are shown in Fig. 2. It is notable that whereas the slippery sites and stems of the motifs are highly conserved, the lengths of gap3, which are not expected to play a critical role, are variable in both of these examples. Thus it appears that the biologically important elements of the frameshift signals have been conserved, while the unimportant elements have been allowed to drift.

Mutations that have been linked to inherited human diseases correlate with those that are predicted to abolish -1 ribosomal frameshifting.

If programmed -1 ribosomal frameshifting has a biologically relevant function in cellular gene expression, then there should be a correlation between mutations that disrupt frameshifting by altering the -1 ribosomal frameshift signal, and human alleles that have been linked to genetically inherited diseases. This hypothesis predicts that the disease alleles would encode missense mutations, or the addition or deletion of entire codons. A preliminary analysis of the human motif hit database identified four alleles of three genes that fit these criteria (Table 3).

Table 3: Three Human Genes Where Specific Mutations in the Consensus -1 Ribosomal Frameshifting Signals Have Been Linked to Disease.

Description	**Diseases and allelic variants*.**
ETFA-electron transfer flavoprotein α-subunit precursor	Type II glutaricaciduria. Note: allelic variant .0004 (Val270DEL3bp) disrupts the spacing between the slippery site and the RNA pseudoknot.
Triacylglycerol lipase	Lipoprotein Lipase Deficiency. Note: allelic variant .0027(Arg75Ser) disrupts stem 1 of the RNA pseudoknot. Familial Chylomicronemia Syndrome. Note: allelic variant .0021 (Trp86Arg) disrupts stem 2 of the RNA pseudoknot.
FASL receptor	Autoimmune lymphoproliferative syndrome. Note: allelic variant .0007 (Tyr216Cys) disrupts stem2 in the RNA pseudoknot.

**The human diseases that are known to be linked to these genes. References to these can be found in the Online Mendelian Inheritance in Man (OMIM) database on the WorldWideWeb.*

In the human gene encoding triacylglycerol lipase, the .0027 allelic variant of triacylglycerol lipase (linked to lipoprotein lipase deficiency) (Wilson et al., 1993), and the .0021 allelic variant (linked to Familial Chylomicronemia Syndrome) (Gotoda et al., 1992) are both predicted to disrupt the RNA pseudoknot component of the consensus -1 ribosomal frameshift signal. Similarly, the .0007 allelic variant of the FASL antigen (linked to autoimmune lymphoproliferative syndrome) (Bettinardi et al., 1997) is also predicted to disrupt the RNA pseudoknot. Disruption of the mRNA pseudoknot is predicted

to abolish programmed -1 ribosomal frameshifting (reviewed in Dinman, 1995; Jacks, 1996; Farabaugh, 1996; Brierley, 1995; Gesteland and Atkins, 1996; Dinman et al., 1998; TenDam et al., 1990). In addition, the .0004 allele of the ETFA-electron transfer flavoprotein α-subunit precursor (linked to type II glutaricaciduria) (Freneaux et al., 1992) disrupts the spacing between the slippery site and the RNA pseudoknot, which is predicted to result in a decrease in programmed -1 ribosomal frameshifting efficiency (Dinman and Wickner, 1992; Brierley et al., 1991; Brierley et al., 1992; Morikawa and Bishop, 1992).

In summary, a computer implemented method has been developed that is capable of detecting known viral -1 ribosomal frameshift signals. We have demonstrated that these motif hits occur with frequencies approximately one order of magnitude greater than random in many large DNA sequence databases, and there are examples where the consensus frameshift signals appear to be evolutionarily conserved in homologous genes in different organisms. Finally, three examples are shown where single missense mutations that occur in the frameshift signal correspond with previously identified genetically inherited diseases in humans.

Computer identified motif hits can promote efficient levels of programmed -1 ribosomal frameshifting in *S. cerevisiae*.

Using a series of frameshift reporter plasmids and yeast strains previously developed, a set of motif hits that were identified by the computer program were tested for ability to promote efficient levels of programmed -1 ribosomal frameshifting in intact cells. Plasmids to monitor programmed ribosomal frameshifting were previously described (Cui et al., 1996; Dinman et al., 1997;m Dinman and Kinzy, 1997; Tumer et al., 1998; Cui et al., 1998). Briefly, in all of these plasmids, transcription is driven from the yeast PGK1 promoter into an AUG translational start site. The *E. coli lacZ* gene serves as the reporter, and transcription termination utilizes the yeast PGK1 transcriptional terminator. In the p0 plasmids, lacZ is in the 0-frame with respect to the translational start site, and measurement of β-galactosidase activity generated from cells transformed with these plasmids serve as the 0-frame controls. In the p-1 series, the predicted programmed -1 ribosomal frameshift signals have been cloned into unique Bam HI and Sma I sites in p0. Thus, in the p-1 series of plasmids, lacZ is in the -1 frame with respect to the translational start site, and is 3' of a predicted programmed -1 ribosomal frameshift signal such that β-galactosidase can only be produced as a consequence of a programmed -1 ribosomal frameshift. p0 and p-1 are introduced into yeast cells in parallel, and the amount of the lacZ gene product (β-galactosidase) present in both sets of cells are determined. Motif hits amplified by PCR from yeast genomic DNA were cloned into pJD160 in such a way that a programmed -1 ribosomal frameshift is required for translation of the *lacZ* gene. This set constitutes the frameshift test plasmids. Programmed -1 ribosomal frameshift efficiencies were calculated by dividing the β-galactosidase activities generated

from cells harboring frameshift test plasmids by the β-galactosidase activity generated by the 0-frame control, pJD160. As a control to determine the background levels of unprogrammed -1 frameshifting, β-galactosidase activities generated from cells harboring pJD160.-1 were determined. Further, the efficiency of programmed -1 ribosomal frameshifting as promoted by the L-A virus frameshift signal was determined in order to compare the frameshift promoting abilities of the motif hits to a known programmed -1 ribosomal frameshift signal. The results of these experiments demonstrate that the motif hits that were tested are all capable of promoting efficient programmed -1 ribosomal frameshifting as compared to the L-A frameshift signal (Table 4).

Table 4. Motif hits can promote efficient levels of programmed -1 ribosomal frameshifting in intact yeast cells.

Frameshift signal	% -1 ribosomal frameshifting
L-A dsRNA virus	1.9%
RAS1	4.4%

Discussion

Following the hypothesis that biological systems tend to conserve usable regulatory mechanisms, a computer program was developed based on an algorithm describing a set of consensus programmed -1 ribosomal frameshift signals. It has been demonstrated 1) that the program is capable of finding known frameshift signals, 2) that these motif hits occur in the large DNA databases at frequencies that are significantly greater than random, 3) that very similar motif hits can be found to be evolutionarily conserved in homologous genes from different species, 4) that known missense alleles that have been linked to human diseases are predicted to disrupt frameshift signals, and 5) that at least one motif hit from the yeast *S. Cerevisiae* genome is capable of promoting efficient levels of programmed -1 ribosomal frameshifting. These findings indicate that, in addition to viruses, programmed -1 ribosomal frameshifting is also utilized to regulate the expression of chromosomally encoded genes in eukaryotes.

Possible regulatory roles of programmed -1 ribosomal frameshifting.

There are three possible translational outcomes of a programmed ribosomal frameshift. A frameshift could result in the production of an extended fusion protein such as the viral gag-pol protein. In the context of cellular proteins, there are many imaginable consequences of the addition of a C-terminal domain. For example, such a domain could provide a means to physically localize the protein to a different compartment. An additional C-terminal domain could encode an enzymatic or signaling function, or even provide an autoregulatory function. A programmed ribosomal frameshift could also

result in the production of two proteins having identical N-terminal domains and different C-termini. In addition to the consequences listed above, such an outcome could also result in a bifurcation function. For example, the two proteins could have identical input functions (e.g. can both act as a receptor for the same ligand), but different output functions (e.g. transduction of the signal to different regulatory pathways). Thus, programmed ribosomal frameshifting could be utilized by cells to effect activity in different biological regulatory pathways.

A third possible outcome is that programmed ribosomal frameshifting results in a premature termination event. Such an event may signal to the translational complex that the mRNA being translated contains a nonsense mutation. mRNAs which contain nonsense mutations are rapidly degraded via the nonsense-mediated mRNA decay (NMD) pathway (reviewed in Weng et al., 1997). The rate of mRNA decay plays an important role in the regulation of gene expression, and the decay rate of an mRNA can be modulated, depending on the cell type, stage of the cell cycle, or environmental conditions (see Atwater et al., 1990; Cleveland and Yen, 1989; Peltz et al., 1991 for reviews). It has been shown that aberrant regulation of post-transcriptional control mechanisms can lead to disease (reviewed in Ross, 1995). Altered stability of certain mRNAs has been suggested to be an important factor in determining the onset and severity of disease. Examples include the differences in the stability between the wild-type c-*myc* mRNA and its tranlocated formed found in Burketts lymphoma; between the highly oncogenic v-*fos* mRNA and its weakly oncogenic c-*fos* mRNA (reviewed in Weng et al., 1997; Lee et al., 1988; Raymond et al., 1989) and between mRNAs encoding the oncogenic E6/E7 proteins of the nonintegraed human papilomaviruses found in benign cervical lesions and the more stable E6/E7 mRNAs synthesized from the integrated form of the virus that correlates with cervical carcinomas (Jeon and Lambert, 1995). Further, mutations in *trans*-acting factors that regulate mRNA turnover may also lead to aberrant gene regulation and disease. Mutations in *trans*-acting factors specifically stabilize the lymphokine GM-CSF mRNA in monocytic tumors compared with non-tumor cells (Schuler and Cole, 1988).

As noted above, both the *RAS1* and STE5 programmed ribosomal frameshift signals fall into this class, promoting approximately 5% of translating ribosomes to encounter premature termination signals. One concern is the biological significance of a mere 5% efficiency of frameshifting in that is this would result in an insignificant 5% change in overall Ras1 protein concentrations. However, this does not take into account the fact that a -1 ribosomal frameshift would lead to the premature translational termination of that specific mRNA molecule. As such, a frameshift event on a specific mRNA would trigger the destruction of that mRNA, and thus these frameshift signals should act as mRNA destabilizing elements, decreasing the overall stability of all of those mRNAs. For example, in the absence of a frameshift signal, each mRNA might be translated 100 times, resulting in the production of 100 protein molecules per mRNA. In the presence of the signal however, a frameshift efficiency of 5% would result in 1 in 20

translating ribosomes encountering a premature termination signal on each individual mRNA, activating NMD pathway. Thus, each mRNA would be limited to producing an average of only 19 of 20 protein molecules, an 80% reduction in the total amount of protein synthesized. Thus we propose that programmed ribosomal frameshifting may be used by a subset of cellular mRNAs as a general mechanism to regulate their stability and consequently the abundance of their encoded protein products.

The abundance of a subset of cellular mRNAs may be biologically regulated by modulation of programmed -1 ribosomal frameshifting efficiencies. As noted above, the rate of mRNA decay plays an important role in the regulation of gene expression, and the decay rate of an mRNA can be modulated, depending on the cell type, stage of the cell cycle, or environmental conditions. Thus, programmed -1 ribosomal frameshifting may be used as a mechanism to regulate the abundance of a subset of cellular mRNAs. The possibilities for signaling mechanisms that may act to modulate programmed -1 ribosomal frameshift efficiencies are numerous. These may include the cell-cycle, heat shock, and developmental, and other signals.

The recent observation that anisomycin specifically inhibits programmed -1 ribosomal frameshifting (Dinman et al., 1997) provides a potentially intriguing link between regulation of programmed ribosomal frameshifting and the control of cell growth and division. There is a considerable body of literature describing the ability of anisomycin to activate the Jun kinase/stress-activated protein kinase (JNK/SAPK) pathway (reviewed in Shu et al., 1996; Moxham et al., 1996). Anisomycin stimulates expression of the c-*jun*, c-*fos* and c-*myc* proto-oncogenes (Yu et al., 1996; Moxham et al., 1996; Kawasaki et al., 1996; Hazzalin et al., 1996), activates the MAP-kinases (Moxham et al., 1996; Hazzalin et al., 1996; Nahas et al., 1996; Cano et al., 1996), pre-ribosomal S6, histone H3 and HMG-14 (Hazzalin et al., 1996), ELAM-1 (Gersa et al., 1992), angiotensin II (Yu et al., 1996), the Ras-dependent and Ras-independent pathways (Kawasaki et al., 1996), p38/RK (yeast Hog1p) (Nahas et al., 1996; Cano et al., 1996), MEK6 (Stein et al., 1996), and insulin-like growth factor II (Nielsen et al., 1995). The effects of anisomycin are specific: other protein synthesis inhibitors (*e.g.* cycloheximide or emetine) block cell cycle progresssion without strong JNK/SAPK induction (Shu et al., 1996).

Anisomycin inhibits protein translation at the level of elongation. It has been proposed that inhibition of protein synthesis leads to a decrease in the levels of labile negative growth regulating proteins, thus promoting cell growth and division (Gersa et al., 1992; Smailov et al., 1993; Rosenwald et al., 1995; Abdelmajid et al., 1993). According to this hypothesis however, any general inhibitor of translation should result in this effect, and thus the JNK/SAPK pathway should be nonspecifically induced by any inhibitor of protein synthesis. This is not the case since 1) not all translational inhibitors stimulate this pathway, and 2) pathway-specific induction is observed. Since anisomycin decreases the efficiency of programmed -1 ribosomal frameshifting efficiencies, it is believed that the regulation of expression of proteins involved in the JNK/SAPK signaling pathway occurs at the post-transcriptional

level by regulating efficiencies of ribosomal frameshifting rather than by generally inhibiting protein synthesis. This model retains the suggestion that there is a labile element tied to specific inhibitors of protein synthesis, but that it is mRNA instead of protein. Thus, anisomycin likely causes an increase in the abundance of these labile cellular mRNAs which encode positive growth regulators by decreasing programmed ribosomal frameshifting efficiencies. In normal growth these mRNAs would promote ribosomes to shift reading frame into early termination codons, making these mRNAs substrates for the nonsense-mediated mRNA decay pathway. These mRNAs would normally be non-abundant species with short half-lives and low production of their encoded protein products. However, under certain conditions, they could be stabilized as a consequence of decreased efficiencies of ribosomal frameshifting. Stabilization of these mRNAs would upregulate the expression of their encoded products, which presumably are positive regulators of cell growth and division. The ability to specifically regulate the half-lives, and thereby the abundance, of mRNAs containing -1 ribosomal frameshift signals provides the cell with a level of specificity that the labile negative growth regulating protein model cannot account for.

Several lines of evidence are consistent with this model. First, anisomycin should stabilize nonsense-mRNAs. It has been demonstrated that anisomycin acts post-transcriptionally by stabilizing the ELAM-1 mRNA and other nonsense-containing mRNAs (Gersa et al., 1992; Li et al., 1996), and that anisomycin regulates the expression of prepro-IGF-II in a post-transcriptional manner (Nielsen et al., 1995). Second, if anisomycin induces cell proliferation by decreasing -1 ribosomal frameshifting efficiencies in a specific set of mRNAs, then sparsomycin should have anti-proliferative effects by virtue of its ability to increase -1 ribosomal frameshifting efficiencies (see Dinman et al., 1997). Sparsomycin analogs have been demonstrated to have antitumor activities (Hofs et al., 1995a; Hofs et al., 1995b; Hofs et al., 1994). Third, in three of the well characterized examples of non-viral programmed ribosomal frameshifting, all involve autoregulatory feedback mechanisms where levels of the encoded protein products affect the efficiencies of ribosomal frameshifting along their own mRNAs (reviewed in Gesteland and Atkins, 1996). These examples where ribosomal frameshifting efficiency is autoregulated provide further support for the hypothesis that programmed ribosomal frameshifting can be used to regulate the abundance and expression of cellular mRNAs and their encoded products.

All of the publications cited herein or listed below are cited for background purposes and the disclosure of such publications is not essential for an understanding of the invention. All of the publications are hereby incorporated by reference.

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What is claimed is:

1. A method of identifying a nucleic acid sequence involved in ribosomal frameshifting comprising:

1) searching a database of gene sequences to identify sequences which contain the sequence XXX YYY Z, wherein

XXX represents GGG, AAA, TTT or CCC,

YYY represents AAA or TTT,

Z represents A, T, or C

and wherein XXXYYYZ is not AAAAAAA or TTTTTTT;

2) further searching among those sequences identified in step 1 for a sequence encoding a pseudoknot structure which is within eight nucleotides of the sequence identified in step 1.

2. The method of claim 1, wherein XXXYYYZ represents a sequence selected from the group consisting of GGG AAA A, GGG AAA T, GGG AAA C, AAA AAA T, AAA AAA C, TTT AAA A, TTT AAA T, TTT AAA C, CCC AAA A, CCC AAA T, CCC AAA C, GGG TTT A, GGG TTT T, GGG TTT C, AAA TTT A, AAA TTT T, AAA TTT C, TTT TTT A, TTT TTT C, CCC TTT A, CCC TTT T, and CCC TTT C.

3. A method of identifying a nucleic acid sequence involved in ribosomal frameshifting comprising the steps of:

selecting a gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC;

selecting said gene sequence having an adjacent sequence of nucleotides from the group of AAA and TTT;

selecting said gene sequence having a nucleotide from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides;

excluding said gene sequence wherein said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A;

excluding said gene sequence wherein said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T;

searching for an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

4. The method of claim 3 wherein XXXYYYZ represents a sequence selected from the group consisting of GGG AAA A, GGG AAA T, GGG AAA C, AAA AAA T, AAA AAA C, TTT AAA A,

TTT AAA T, TTT AAA C, CCC AAA A, CCC AAA T, CCC AAA C, GGG TTT A, GGG TTT T, GGG TTT C, AAA TTT A, AAA TTT T, AAA TTT C, TTT TTT A, TTT TTT C, CCC TTT A, CCC TTT T, and CCC TTT C.

5. A system for identifying a nucleic acid sequence involved in ribosomal frameshifting, the system comprising:

access means for accessing a database of gene sequences;

selection means for selecting a particular gene sequence from said database of gene sequences, said particular gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC, an adjacent sequence of nucleotides from the group of AAA and TTT, a nucleotide from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides, wherein said particular gene sequence is excluded from selection when said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A and said particular gene sequence is excluded from selection when said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T;

pseudoknot search means for locating an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

6. The system as recited in claim 5 wherein XXXYYYZ represents a sequence selected from the group consisting of GGG AAA A, GGG AAA T, GGG AAA C, AAA AAA T, AAA AAA C, TTT AAA A, TTT AAA T, TTT AAA C, CCC AAA A, CCC AAA T, CCC AAAC, GGG TTT A, GGG TTT T, GGG TTT C, AAA TTT A, AAA TTT T, AAA TTT C, TTT TTT A, TTT TTT C, CCC TTT A, CCC TTT T, and CCC TTT C.

7. A method of regulating expression of a mammalian gene comprising modulating the frequency of ribosomal frameshifting during translation of messenger RNA.

8. The method according to claim 7, wherein the frequency of frameshifting is increased.

9. The method according to claim 7, wherein the frequency of frameshifting is decreased.

10. The method according to claim 7, wherein the gene encodes an oncogene.

11. The method according to claim 7, wherein the gene encodes a tumor suppresser gene.

12. The method according to claim 7, wherein the gene encodes a hormone.

13. The method according to claim 7, wherein the gene encodes a human growth hormone.

14. The method according to claim 7, wherein the gene encodes a hormone receptor.

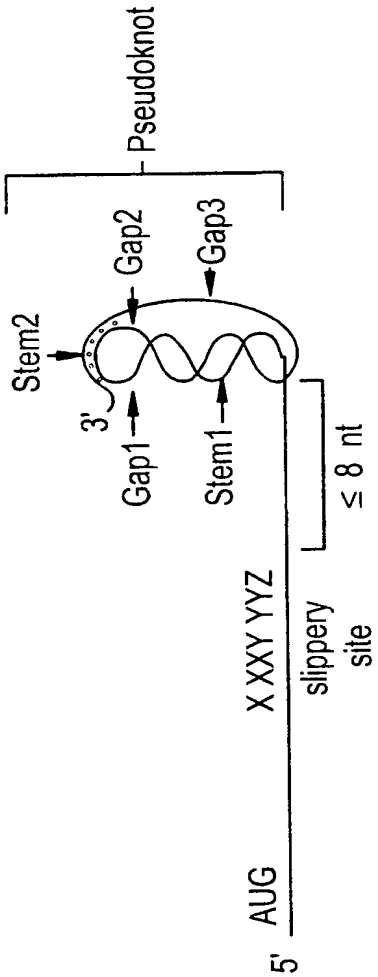
15. The method according to claim 7, wherein the gene encodes a human growth hormone receptor.

16. The method according to claim 6, wherein the gene encodes a catalytic enzyme.

17. A method of treating a disease caused by reduced expression of a gene product which is produced as a result of ribosomal frameshifting, comprising increasing the frequency of ribosomal frameshifting during translation of the gene.

18. A method of treating a disease caused by increased expression of a gene product which is produced as a result of ribosomal frameshifting, comprising decreasing the frequency of ribosomal frameshifting during translation of the gene.

FIG. 1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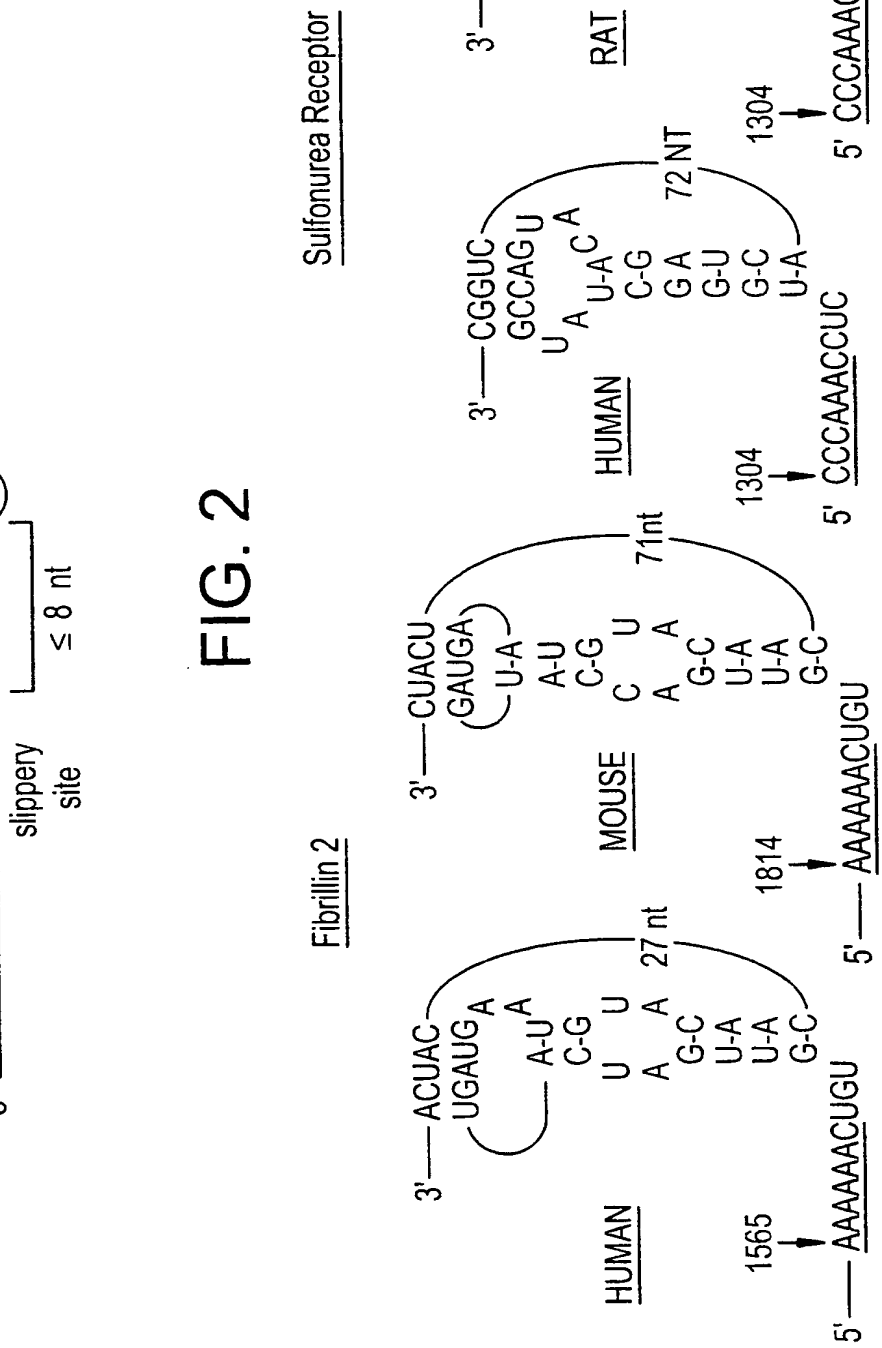
slippery site

FIG. 2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MOUSE
RAT
HUMAN
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UGAUG
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C-G
U U
A A
G-C
U-A
U-A
G-C
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3'—CUACU
GAUGA
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20942

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 15/11, 15/12
US CL : 435/6, 440; 536/23.1, 23.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 440; 536/23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, Biosis, CAPlus, Derwent WPI, USPAT
search terms: ribosomal frameshift, search, identify, computer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MATSUFUJI et al. Autoregulatory Frameshifting in Decoding Mammalian Ornithine Decarboxylase Antizyme. Cell. 13 January 1995, Vol. 80, pages 51-60, especially page 51.	7-9
Y	HONDA et al. RNA Signals for Translation Frameshift: Influence of Stem Size and Slippery Sequence. Biochem. Biophys. Res. Commun. 1995, Vol. 213, No. 2, pages 575-582, especially page 575.	1-9
Y	LEE et al. Identification of a Ribosomal Frameshift in Leishmania RNA Virus 1-4. J. Biochem. 1996, Vol. 120, pages 22-25, especially page 22.	1-6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
29 DECEMBER 1999

Date of mailing of the international search report
03 FEB 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20942

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHEFFTER et al. Complete Sequence of Leishmania RNA Virus 1-4 and Identification of Conserved Sequences. Virology. 1994, Vol. 199, pages 479-483, especially page 483.	1-6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20942

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20942

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group 1, claim(s)1-6, drawn to a method of identification of a ribosomal frameshift sequence.
Group 2, claim(s) 7-18, drawn to a method of modulating gene expression.

The inventions listed as Groups 1 and 2 do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature of Group 1 is a method of identifying a nucleic acid sequence involved in ribosomal frameshifting. The special technical feature of Group 2 is a method of regulating expression of a gene by modulating the frequency of ribosomal frameshifting. Therefore the two groups have different special technical features.